ISSN (print) 0093-4666 © 2012. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/122.271

Volume 122, pp. 271–282 *October–December 2012*

Development and morphology of *Lysurus cruciatus* — an addition to the Indian mycobiota

S. Abrar, S. Swapna & M. Krishnappa*

Department of Post Graduate Studies and Research in Applied Botany, Kuvempu University, Shankaraghatta-577451, Karnataka, India

* Correspondence to: *krishnappam1007@gmail.com*

Abstract — *Lysurus cruciatus* (*Phallomycetidae*, *Phallaceae*) produces a basidiome with a long receptacle terminating in conical arms that branch initially and interconnect at their tips to support gleba on lateral surfaces. Its development from primordia to mature basidiome has been microscopically examined. Fructification begins as a small protuberance that differentiates into a series of layers and with gelatinization develops into an egg. The matured fruitbody stands up with an expanded receptacle. *Lysurus cruciatus* is a new record for the India mycobiota.

Key words — hymenium, intermediate tissue, medulla, ontogeny, peridial tissues

Introduction

Although morphology and taxonomy of phalloids have been frequently studied throughout the last century, their developmental characters have rarely been examined (e.g., Burt 1896, Fischer 1910a, Atkinson 1911, Petch 1911, Cleland & Cheel 1917, White 1944, Flegler & Hooper 1980, Sáenz et al. 1983, Zang & Peterson 1989, Abrar et al. 2007). In India, studies on *Phallaceae* associated with basidioma development are very scanty as previous reports were mainly focused on documentation (Narasimhan 1932, Ahmed 1939, Apte 2005), and there are no reports of *Lysurus* from India (Jamaluddin et al. 2004).

The tropical genus *Lysurus* is saprobic (Schaffer 1975, Lodge & Cantrell 1995) and morphologically characterized by short columnar arms (Teng 1996) attached to the apical margin of a cylindrical receptacle (Fischer 1886) that emerges from a ruptured peridium (Zang & Peterson 1989). The gleba, which is located on the lateral surfaces of receptacle arms (Miller et al. 1991, Beltrán-Tejera et al. 1998, Cortez et al. 2011), emits a fetid odor (Burk et al. 1982). *Lysurus cruciatus* is commonly known as lizard's claw stinkhorn in U.S.A. (Arora 1986).

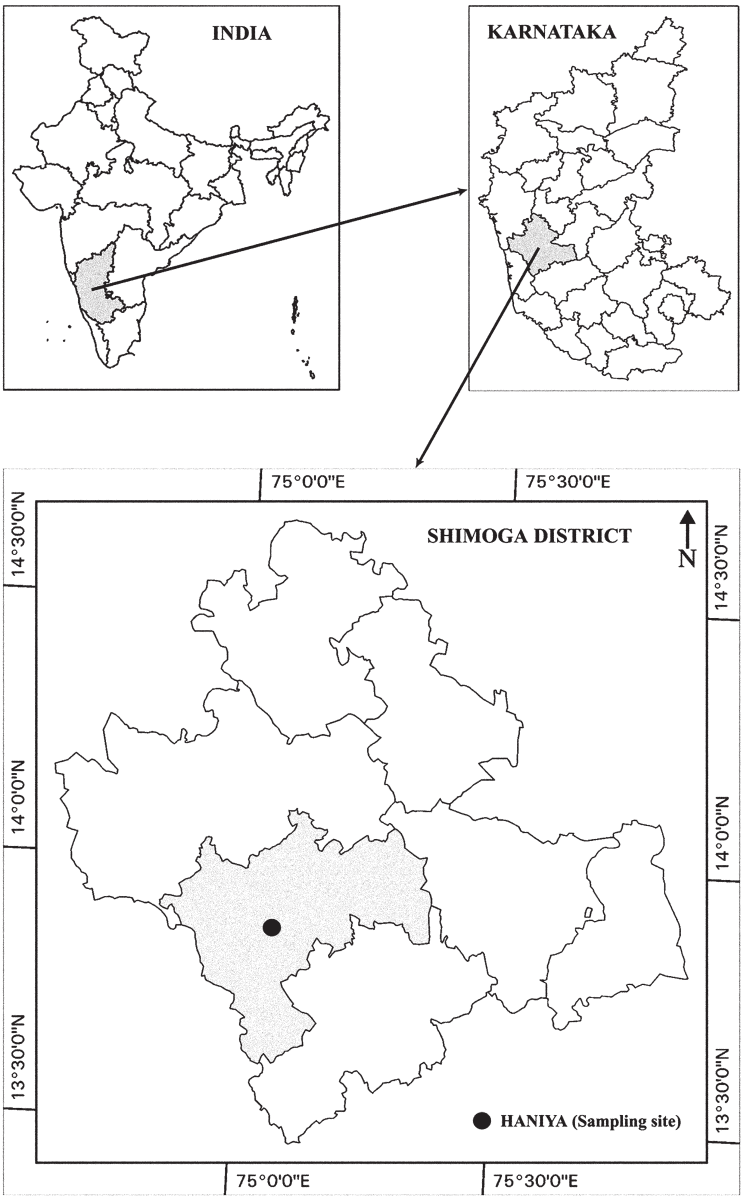


Fig. 1. *Lysurus cruciatus* sampling location in Haniya, Shimoga district, India.

In this report of a new record of *L. cruciatus* from the Indian subcontinent, we focus on the developmental stages of the fruitbody.

Materials & methods

Primordia and sequentially matured basidioma of *L. cruciatus* were collected from a site in the Western Ghats, Karnataka (Fig. 1). Macroscopic characters were described from observations of fresh material; color notations are from Kornerup & Wanscher (1981). The primordia from different stages were fixed in Pfeiffer's solution containing absolute methanol and 40% formalin (w/v) in equal proportions. All measurements were made in 3% KOH. Free hand sections of primordial stained with 1% lactophenol cotton blue and 1% phloxine were mounted on glass slides and examined under the stereo and optical microscopes. Cited collections are deposited in the Applied Botany Herbarium, Kuvempu University, Shankaraghatta, Shimoga District, Karnataka, India (KUABSAK).

Taxonomic description

Lysurus cruciatus (Lepr. & Mont.) Henn., Beibl. Hedwigia 41: 172, 1902 Figs. 2, 3a
MycoBank 100531

Immature Fruit Bodies ('eggs') 45–60 mm in diam., spherical to obovoid, white (1A1) always arising from white (1A1) mycelium cord buried under the soil. Receptacle consisting of a stipe and apical branches or arms. Stipe 85–100 × 20–25 mm, cylindrical, narrowly obconical, hollow, 3–4 mm thick, longitudinally grooved and transversely ribbed, white (1A1), composed of a pseudoparenchyma with cells of 19.2–30.5 × 17–31.6 µm, globose to subglobose. Receptacle arms 25–40 × 6–8 mm (lengths variable within the same sporome), 4–6 (usually 5), conical, constricted at the top, hollow, a deep marked groove running along the whole abaxial surface of arms, transversely wrinkled in outer surface, initially fused at their tips, then free tending to curve slightly away from the axis of the receptacle, outer concave surface smooth, white, inner convex surface transversely rugulose, with pseudoparenchyma cells of 16.5–25.6 µm diam., globose to subglobose. Gleba in the inner convex surface of the arms, brown to grayish brown (6E2-6E3), gradually deliquescing, unpleasant stinkhorn odor. Volva 35–40 × 20–25 mm, saccate, thick, white (1A1), with aerolations, composed of scattered hyphae 2.4–3.6 µm wide, stipe and volva separated by a clear jelly. Mycelial cords proliferating from the base, several white. Basidiospores 4–4.5 × 1.5–2 µm, elliptic to oblong, thin-walled, smooth, hyaline.

Ecology & Distribution: Gregarious growing in areca nut plantations in the tropical zone. Fruiting from July to October with a peak in August. Also known from America, Asia, Australia, Africa. Introduced in Europe.

Specimens examined – **India**, **Karnataka**, Western Ghats, Shimoga district, Haniya, 4 km from Hosanagar taluk, 13°52′58″N 75°03′27″E, alt. 742 m, S. Abrar, S. Swapna, &

M. Krishnappa, 28.VII.2007 (KUABSAK-MSH10); 21.VII.2008 (KUABSAK-MSH30); 08.X.2009 (KUABSAK-MSH102).

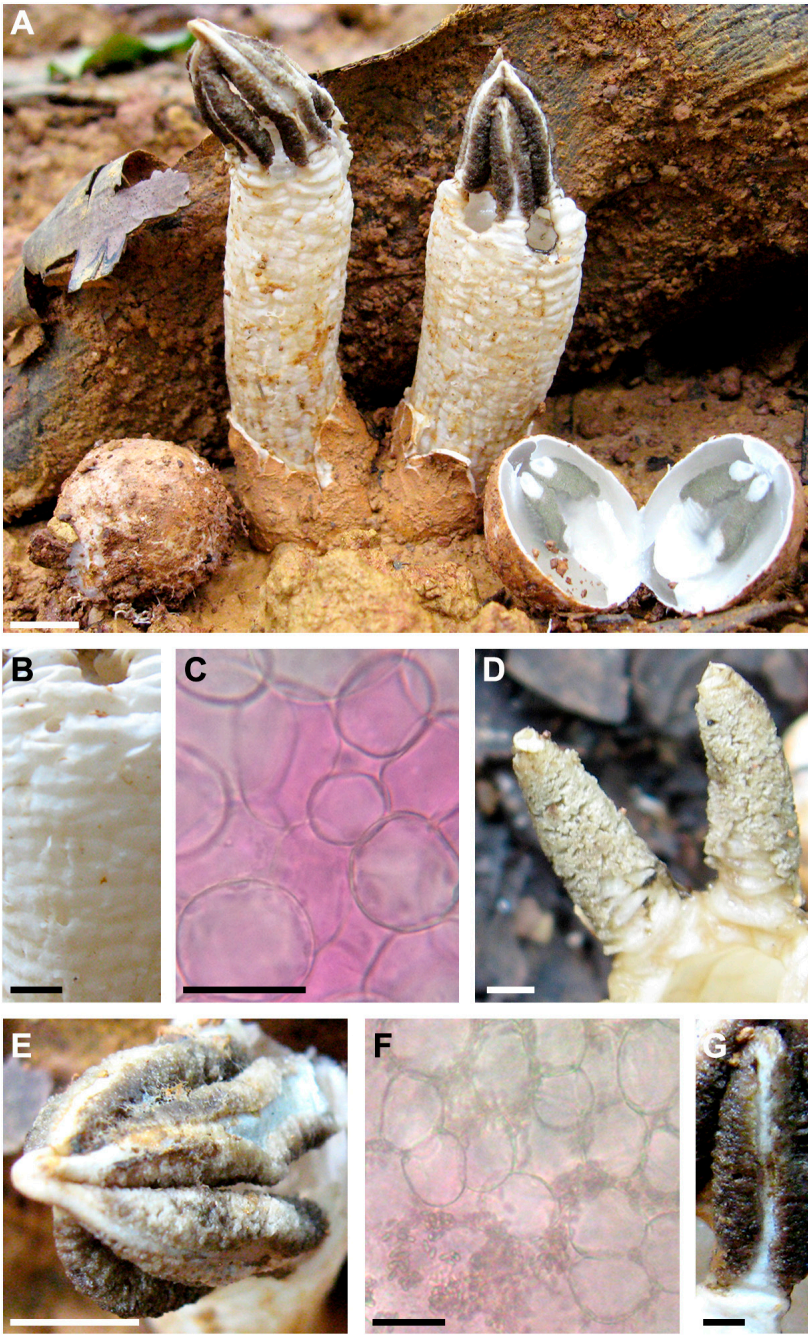
Developmental stages FIGS. 3B–F, 4

EGG PHASE: Initially small (0.5 mm diam.) white (1A1) swellings form at the end of mycelial strands. The 1–2 mm diam. primordia or young sporoma (YS) differentiate into a central medulla (M) and peripheral cortex (C). As differentiation continues, primary volva-gel (VG) forms from the medulla while the cortical cells fold inward (IF) four to six times. These infoldings differentiate and enlarge into lobes (L). The enlarging lobes gradually compress cortical cells to form a layer of intermediate tissue (IT) 2–4 mm in diam. The infoldings or clefts (CF) increase at the junction of intermediate tissue and medulla with the differentiation of peridial plates (PP) (4–5 mm in diam.). The external medullary region differentiates into palisade tissue (PT), comprising the primordium of the gleba (G) and develops sequentially to form enlarged clefts (ECF) (5–6 mm diam). Intermediate tissue continuously thickens along the clefts to initiate formation of receptacle arms (IRA) while the medullary tissue thickens to form the initials of the receptacle (IR) (6–8 mm diam.). The gleba enlarges (EG) (8–10 mm diam.) with the complete transformation of palisade tissue. The gleba primordium further differentiates hyphae that form the evenly arranged probasidial tips and later matures to form hymenium and trama layers. The continuous glebal development along distorted peridial plates breaks the volva-gel, which corresponds to receptacle arms (RA) (10–20 mm in diam.). The gleba gelatinization (GG) and the development of pseudoparenchymatous tissue of receptacle (R) (20–40 mm diam.) increases the size of the egg. The gleba is then reduced (RG) as grooves (GR) form outside each receptacle arm (40–45 mm diam.). Maturation proceeds with further differentiation of pseudoparenchyma in the receptacle wall and receptacle arms and with the continuous production of basidiospores from the pseudohymenial layer.

During development, the primordial cortex gives rise to the outer skin (peridium), peridial plates, and receptacle, while the volva-gel and gleba derive from the medulla. Before rupture, the mature egg differentiates into outer skin and inner volva-gel that encloses the gleba, receptacle, and receptacle arms. Complete development and differentiation of the various egg parts up through basidiospore formation occurs in 6–13 days.

After final internal development (basidiospore production), eggs become epigeous from an initial hypogeous state. Before rupture, the pre-differentiated

FIG. 2. *Lysurus cruciatus* (KUABSAK-MSH 30). A, Mature receptacle. B, Receptacle with transverse and longitudinal grooves. C, Pseudoparenchymatous tissue of receptacle. D, A groove underneath arm. E, Arms united at their tips. F, Pseudoparenchymatous tissue of an arm. G, An arm with external longitudinal furrow. Scale bars: A, E= 1cm; B, D = 5mm; C = 30µm; F = 20 µm; G = 3mm.


A
B
C
D
E
F
G

sporome within the egg is surrounded by a pale grayish gelatinous mucilage. A crack occurs, tearing at the apical portion (corresponding to the tip of the arms) of the egg.

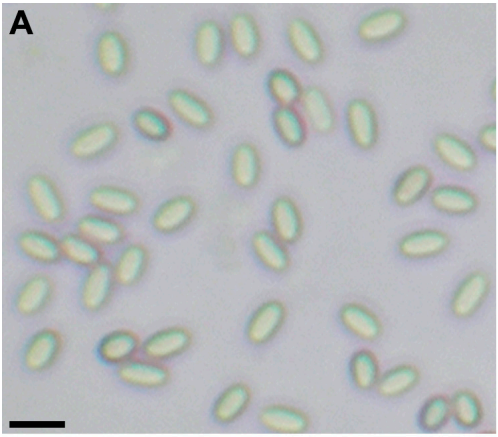
Receptacle phase: The receptacle elongates through the crack due to osmosis and cell elongation. The receptacle expands freely in 2–4 seconds with the autolysis of the gleba upon the receptacle arms (closely compressed together in the egg stage). The peridium is retained as a volva with mycelial strands at the base. The arms are set free with the moistening of the gleba, after which the gleba deliquesces and becomes fetid (Fulton 1889). Insects especially associated with carrion are attracted towards the sticky gleba. The mature phalloid basidiome, which lasts for 20–34 hrs, then autodigests and disintegrates. Under favorable environmental conditions, the dispersed basidiospores germinate to form mycelium. Development proceeds with the formation of eggs and the life cycle continues.

Discussion

Although Corda (1842) placed the genus *Lysurus* in a new family, *Lysuraceae* (as "*Lysuroideae*"), which he separated from *Clathraceae,* many 20th century mycologists treated *Lysuraceae* under *Clathraceae* (Cunningham 1944, Dring 1980, Zeller 1949). Fungal classification resulting from the Assembling the Fungal Tree of Life (AFTOL) project revealed *Phallaceae* and *Clathraceae* as separate families with *Lysuraceae* more closely related to *Phallaceae* than to *Clathraceae* (Hosaka et al. 2006). *Lysuracea*e fruitbodies resemble *Clathraceae* in having sutures dividing the gelatinous layer but differ in the long receptacles that are stipitate, longer than the arms arising from the receptacles, and with a gleba always tending to migrate to the exterior part of the arms (cf. the gleba in *Clathraceae* being attached only to the interior face of the arms). Currently, *Lysurus* is placed under *Phallaceae*, order *Phallales*, subclass *Phallomycetidae*. The phylogenetic position of *Phallales* indicates that that stinkhorns are derived from truffle-like fruitbodies (Hosaka et al. 2006, Hibbett et al. 2007).

In *Aseroe arachnoidea* E. Fisch., the peridial plates are produced radially from gleba (Fischer 1910b) whereas in *Protubera* sp., *Phallus impudicus* L., and *Ithyphallus tenuis* E. Fisch., the plates develop from the columella (Fischer 1887, Gäumann & Dodge 1928). In *Mutinus bambusinus* (Zoll.) E. Fisch., the plates develop from the apical part of the undeveloped cap (Fischer 1886) and in *Clathrus delicatus* Berk. & Broome the junction of the cortical tissue and clefts give rise to the peridial suture (Swapna et al. 2010). In *Lysurus cruciatus*, it develops from the junction of the intermediate tissue and medulla.

Fig. 3. *Lysurus cruciatus*. A, Basidiospores. B, Epigeous egg. C, D, Crack occurring at the apical portion of the egg. E, Expanded receptacle from egg. F, Insects feeding on gleba. Scale bars: A = 5µm; B, C = 1cm; D–F = 2 cm.


A

B

E

C

D

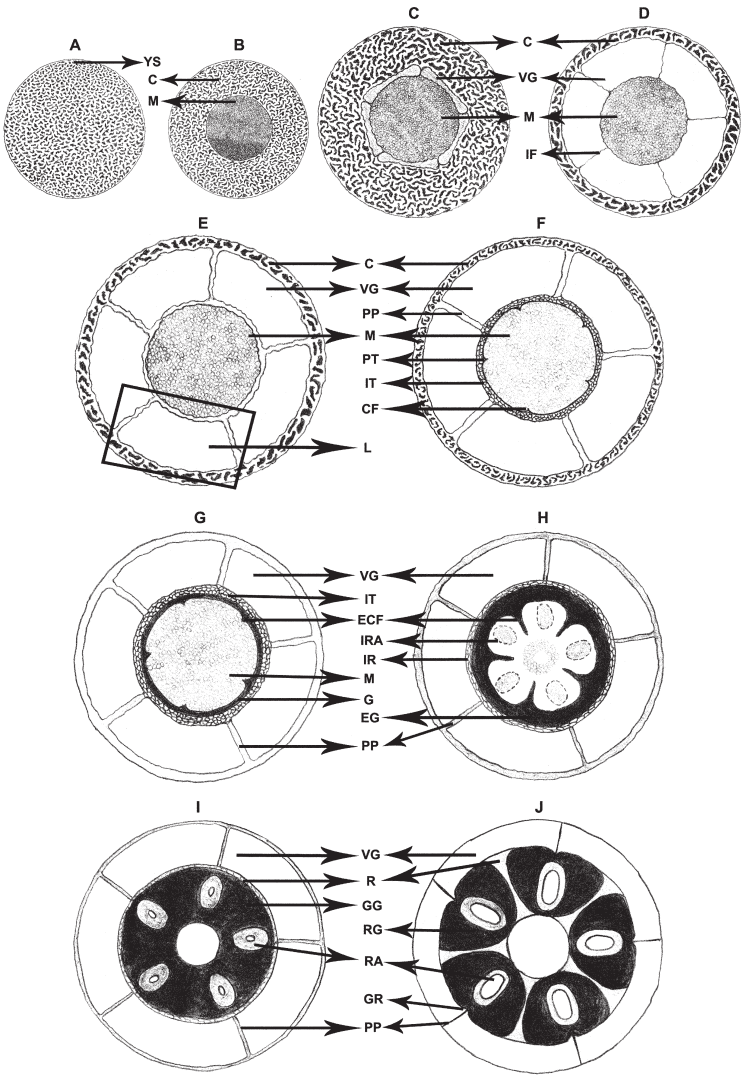
F

In *A. arachnoidea,* jelly begins to form as the stalk primordium develops (Fischer 1910b), while in *Protubera* sp., *Mutinus caninus* (Huds.) Fr., and *I. tenuis* jelly formation begins during formation of the young fruiting body (Gäumann & Dodge 1928; Fischer 1886). In *L. cruciatus* and *C. delicatus,* the jelly is formed by the differentiation of medulla (Swapna et al. 2010).

In *Simblum sphaerocephalum* Schltdl. thick strands of tissue form the central branches that run radially outwards from the center and fuse into volva-jelly at the middle of one of the receptacle meshes (Conard 1913). In *Aseroe arachnoidea* the branches are borne from the central strand (Fischer 1910b) but in *Protubera* sp., are initiated from the columella. In *M. caninus*, the cap forms from a central intertwined portion whereas in *Phallus impudicus*, the cap develops from intermediate tissue (Gäumann & Dodge 1928). In *I. tenuis* the stalk develops before forming a cap at its apex (Fischer 1886). In *C. delicatus*, the branches arise from the hyphal knots (Swapna et al. 2010) whereas in *L. cruciatus* the intermediate tissue constantly thickens to form receptacle arms.

The glebal chambers of *Aseroe rubra* Labill. develop by formation of pseudoparenchyma cells, after which trama plates are fused together and the spore mass develops horizontally around the mouth of the attached stalk (Fischer 1910b). In *M. caninus* the gleba develops during formation of the primordial plates followed by the trama and hymenium (Gäumann & Dodge 1928). In *I. tenuis* the apex of the head wrinkles to form trama wherein it extends as a dense palisade layer and bulges to form the hymenium. Later the trama assumes a gelatinous texture and sporulation initiates (Fischer 1886). In *C. delicatus* the palisade tissue transforms into gelatinized columella and the trama adheres tightly in developing receptacle, before the basidiospores form (Swapna et al. 2010). In *L. cruciatus* the external medullary region differentiates into palisade tissue constituting the glebal primordium. The gleba enlarges with differentiation of the probasidial tips and matures to form pseudohymenium, trama, and basidiospores.

According to Mycobank, six *Lysurus* species are currently accepted. *Lysurus cruciatus* and *L. gardneri* Berk. both produce white to pale cream columnar receptacle, which distinguish them from *Lysurus mokusin* (L.) Fr., characterized by well marked white to reddish angular fluted receptacle. In *L. mokusin,* the receptacle arms lie directly on the glebal columella in contrast to the arms separated with deep grooves in *L. cruciatus. Lysurus periphragmoides* (Klotzsch) Dring is unique having subglobose to ovoid clathrate network of arms with mostly pentagonal to hexagonal meshes that are at first yellow and then orange red (Dring 1980). *Lysurus pakistanicus* S.H. Iqbal et al. resembles *L. periphragmoides* in its white to yellowish hollow, narrowly obconical receptacle but differs from the latter by an irregularly oblong or globose apex formed by



Figs. 4. Sporoma development of *Lysurus cruciatus* (KUABSAK-MCH 30). A, Primordium. B, Differentation of medulla and cortex. C, Differentiation of volva-gel. D, Cortical cells forming infoldings. E, Formation of lobes. F, Formation of intermediate tissue and peridial plates. G, Formation of palisade tissue. H, Initiation of receptacle and receptacle arms. I, Development and gelatinization of gleba. J, Formation of grooves and reduction of gleba. Abbreviations: C = Cortex, CF = Cleft, ECF = Enlarged cleft, EG = Enlarged gleba, G = Gleba, GG = Gelatinization of gleba, GR = Groove, IF = Infolding, IR = Initiation of receptacle, IRA = Initial of receptacle arm, IT = Intermediate tissue, L = Lobe, M = Medulla, PP = Peridial plate, PT = Palisade tissue, R = Receptacle, RA = Receptacle arm, RG = Reduction of glebal mass, VG = Volva-gel, YS = Young sporoma.

a clathrate network of anastomosing arms, irregular to cylindrical with up to 10 meshes that is rosy to orange pink at maturity; in *L. pakistanicus* the gleba fills the entire interior of the arms and extends outwards in the meshes (Iqbal et al. 2006). *Lysurus corallocephalus* Welw. & Curr. is entirely different, with erect appendages that are usually dark or flushed pink above and white to buff or yellowish below and which have simple or forked with slender intersections.

Lysurus cruciatus differs from the other known *Lysurus* species by the uniquely transversely rugulose glebiferous surface. The glebiferous region is villose and lamellate in *L. gardneri,* slightly keeled on both outer and inner surfaces in *L. pakistanicus* and *L. periphragmoides,* and corrugated on the sides and flat on the inner surface *L. periphragmoides* is (Dring 1980, Iqbal et al. 2006). The gleba is on the lateral surface of the arms in *L. mokusin* and distributed on the exterior of polygonal meshes in *L. corallocephalus* (Dring 1980).

In *L. gardneri* the stalk divides at the apex and does not bear any glebiferous layer for 2–4 mm from the base of the arm. Each arm is well marked and borne on its own stalk with a narrow zone, with the sterile base appearing as a furrow down the outer face (Petch 1911). The diameter of the arms at 3 mm above the stalk region increases to 6 mm in the glebiferous region. The gleba of *L. cruciatus* runs along the groove in the intersection without any sterile base. The diameter of the arms is almost the same through its length. The glebal content is comparatively less in *L. cruciatus* compared to *L. gardneri,* which is more concentrated in the center of the arms with a bulging appearance and reduced towards the tips.

Acknowledgment

We are grateful to the University Grant Commission (UGC), New Delhi for funding the project. We also thank Mr. K.N. Shankar Narayan Bhat and Mr. K.S. Vinayaka, from Haniya in Hosanagar Taluk (Karnataka) for their help during the field studies. We thank Drs. Vagner G. Cortez (Universidade Federal do Paraná, Brazil) and Laura Guzmán-Dávalos (Departamento de Botánica y Zoología, Universidad de Guadalajara, México) for critical review of the manuscript.

Literature cited

Abrar S, Swapna S, Krishnappa M. 2007. *Dictyophora cinnabarina*. Current Science 92: 1219–1220.

Ahmed S. 1939. Higher fungi of Punjab plains. Journal of Indian Botanical Society 11: 248–254.

Apte D. 2005. First record of *Clathrus delicatus* Berkeley & Broome (1873) from Sanjay Gandhi National Park. Journal of Bombay Natural History Society 102: 135–136.

Arora D. 1986. Mushrooms demystified: a comprehensive guide to fleshy fungi. Ten Speed Press, California. 959 p.

Atkinson GF. 1911. The origin and taxonomic value of the veil in *Dictyophora* and *Ithyphallus.* Botanical Gazette 50: 1–20 + 7 pl.

Beltrán-Tejera E, Bañares-Baudet A, Rodríguez-Armas JI. 1998. Gasteromycetes of the Canary Islands: some noteworthy new records. Mycotaxon 68: 439–453.

Burk WR, Flegler SL, Hiss WM. 1982. Ultrastructure studies of *Clathraceae* and *Phallaceae* (*Gasteromycetes*) spores. Mycologia 74: 166–168. http://dx.doi.org/10.2307/3792646

Burt EA. 1896. The development of *Mutinus caninus* (Huds.) Fr. Annals of Botany 10(39): 343–372.

Cleland JB, Cheel E. 1917. Notes on the early stage of the development of *Lysurus gardneri* (*Lysurus australiensis*). Journal of Royal Society New South Whales 51: 364–365.

Conard HS. 1913. Structure of *Simblum sphaerocephalum*. Mycologia 5: 264–273. http://dx.doi.org/10.2307/3753269

Corda ACJ. 1842. Icones fungorum hucusque cognitorum.Vol. 5. 92 p

Cortez VG, Baseia IG, Silveira RMB. 2011. Gasteroid mycobiota of Rio Grande do Sul State, Brazil: *Lysuraceae* (*Basidiomycota*). Acta Scientiarum. Biological Sciences 33: 87–92. http://dx.doi.org/10.4025/actascibiolsci.v33i1.6726.

Cunningham GH. 1944. The *Gasteromycetes* of Australia and New Zealand. Dunedin, New Zealand. 236 pp + 36 pl.

Dring DM. 1980. Contributions towards a rational arrangement of the *Clathraceae*. Kew Bulletin 35: 1–96. http://dx.doi.org/10.2307/4117008

Fischer E. 1886. Versuch einer systematischen Übersicht über die bisher bekannten Phalloideen. Jahrbuch des Königlichen Botanischen Gartens und des Botanischen Museums zu Berlin 4: 1–92.

Fischer E. 1910a. Beiträge zur Morphologie und Systematic der Phalloideen. Annales Mycologici 8: 314–322.

Fischer E. 1910b. Die fruchtkörper-entwicklung von *Aseroë*. 595–614, in: Treube MM (eds), Annales du Jardin botanique de Buitenzorg, supp. 3(2) & 4. EJ Brill, Leide.

Flegler SL, Hooper GR. 1980. Ultrastructure and development of *Mutinus caninus* and the occurrence of eight spored basidium. Mycologia 72: 1001–1014. http://dx.doi.org/10.2307/3759739

Fulton TW. 1889. The dispersion of the spores of fungi by the agency of insects with special reference to the phalloidei. Annals of Botany 3: 207–239.

Gäumann EA, Dodge CW. 1928. The comparative morphology of fungi. Mc Graw Hill, New York. 701 pp.

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lücking R, Lumbsch HT, Lutzoni F, Matheny PB, Mclaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Kõljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schüssler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N. 2007. A higher-level phylogenetic classification of the *Fungi*. Mycological Research 111: 509–547. http://dx.doi.org/10.1016/j.mycres.2007.03.004

Hosaka K, Bates ST, Beever RE, Castellano MA, Colgan III W, Domínguez LS, Nouhra ER, Geml J, Giachini AJ, Kenney SR, Simpson NB, Spatafora JW, Trappe JM. 2006. Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass *Phallomycetidae* and two new orders. Mycologia 98: 949–959. http://dx.doi.org/10.3852/mycologia.98.6.949

Iqbal SH, Kasuya T, Khalid N, Niazi AR. 2006. *Lysurus pakistanicus*, a new species of *Phallales* from Pakistan. Mycotaxon 98: 163–168.

Jamaluddin, Goswami MG, Ojha BM. 2004. Fungi of India (1989-2001). Scientific publishers, Jodhpur. 326 pp.

Kornerup A, Wanscher JH. 1981. Methuen handbook of colour, 3rd ed. Eyre Methuen, London. 252 pp.

Lodge DJ, Cantrell S. 1995. Fungal communities in wet tropical forests: variation in time and space. Canadian Journal of Botany 73: 1391–1398. http://dx.doi.org/10.1139/b95-402

Miller OK, Ovrebo CL, Burk WR. 1991. *Neolysurus*: a new genus in the *Clathraceae*. Mycological Research 95: 1230–1234. http://dx.doi.org/10.1016/S0953-7562(09)80016-6

Narasimhan MJ. 1932. The *Phalloideae* of Mysore. Journal of Indian Botanical Society 11: 248–254.

Petch T. 1911. Further notes on *Colus gardneri* (Berk.) Fischer. Transactions of the British Mycological Society 6: 121–132. http://dx.doi.org/10.1016/S0007-1536(17)80020-6

Sáenz JA, Carranza J, Gómez VS. 1983. Estudio comparativo al microscopio de luz y al microscopio electrónico de barrido de *Laternea triscapa*, *Laternea pusilla* y *Ligiella rodrigueziana*. Revista de Biología Tropical 31: 327–331.

Schaffer RL. 1975. The major groups of *Basidiomycetes*. Mycologia 67:1–18. http://dx.doi.org/10.2307/3758222

Swapna S, Abrar S, Manoharachary C, Krishnappa M. 2010. Development and morphology of *Clathrus delicatus* (*Phallomycetidae, Phallaceae*) from India, Mycotaxon 114: 319–328. http://dx.doi.org/10.5248/114.319

Teng SC. 1996. Fungi of China. Mycotaxon, New York. 586 p.

White NH. 1944. The development of *Lysurus sulcatus* (Cooke & Massee). Transaction of the British Mycological Society 27: 29–35. http://dx.doi.org/10.1016/S0007-1536(44)80005-5

Zang M, Peterson RH. 1989. *Endophallus*, a new genus in the *Phallaceae* from China. Mycologia 81: 486–489. http://dx.doi.org/10.2307/3760091

Zeller SM. 1949. Keys to the orders, families and genera of the *Gasteromycetes*. Mycologia 41: 36–58. http://dx.doi.org/10.2307/3755271